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Original Research Paper

Possibility of early detection of graft incompatibility in some commercial plum cultivars by phenolic compounds analysis

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ABSTRACT

The incidence of incompatibility signs in the grafting point can be delayed, and the analysis of phenols is used as an applicable early sign for the detection of graft incompatibility. Accordingly, this study mainly aimed to investigate compatibility/incompatibility in 10 commercial plum cultivars grafted on myrobalan and apricot rootstocks, followed by determining the role of phenols in graft incompatibility. The evaluated cultivars included Santarosa, Ghatreh tala, Shams, Dargazi, No. 16, No. 17, Laroda, Simka, Bokhara, and Stanley. The results showed significant differences in the stem diameter. The union graft location in Shams, Laroda, Simka, Stanley, and Ghatreh tala cultivars on apricot rootstock was thicker than the scions and stocks. Phenolic compounds in the union graft decreased in all plum cultivars on myrobalan rootstock in comparison with other sites. Finally, the most phenolic accumulation belonged to the union graft on Santarosa, Ghatreh tala, and Shams on apricot rootstocks. Therefore, it seems that phenolic compounds in plums can be used as a biochemical marker in graft incompatibility.

Keywords: Apricot rootstock, incompatibility, myrobalan rootstock, phenolic content, plum

INTRODUCTION

Plum (*Prunus* spp.) is one of the most commercially important fruit species in Iran. Plums are temperate zone fruits, but they are widely grown throughout the world, from the cold climate of Siberia to the sub-tropical conditions of the Mediterranean region (Son, 2010). Prunus species such as *P. cerasifera*, *P. domestica*, *P. institia* and *P. Salicina* are widely grown throughout the world. The European plum (*P. domestica*) and the Japanese plum (*P. salicina*) are more important in terms of commercial production (Ozbek, 1978).

Grafting is largely used in the production of vegetable and fruit-bearing crops in order to increase uniformity, vigor, and adaptation to biotic and abiotic stresses. The compatibility of rootstock and scion plays a crucial role in establishing highly efficient root systems through grafting (Goldschmidt, 2014; Warschefsky *et al*., 2016). However, this trait varies significantly even between closely related species, which necessitates the evaluation of compatibility before grafting a specific scion genotype into the rootstock. For the stone fruit industry that heavily relies on vegetatively propagated cultivars (*i.e*., individual genotypes) via grafting, the long-term vitality of the union between the rootstock and scion is crucial (Lee *et al*., 2011; Guan *et al*., 2012).

Graft incompatibility generally occurs at the early stage of graft development when the vascular connection is forming. However, symptoms may manifest at large growth stages such as low plant development related to physiological differences in the stem diameter, which impairs the normal flow of photoassimilates and the lignification of grafted tissues (Souza *et al*., 2018), thus decreasing the hydraulic conductivity of the graft union (Tworkoski and Fazio, 2015). These symptoms appear during the plant fruiting period when the plant is subjected to a high demand for water transport (Martinez-Ballesta *et al*., 2010). Incompatibility does not permanently become

apparent immediately after grafting. It may take several years to manifest failure with establishing graft-union leading to major economic losses to growers and nurseries. In addition, the significant delay in the appearance of incompatibility symptoms renders the evaluation and transfer of new fruit tree genotypes to industry time-consuming, expensive, and laborious (Gainza *et al*., 2015; Pina *et al*., 2017).

Fruit trees are typically formed by a combination of the scion and rootstock. A good union between a scion and rootstock is necessary for a successful combination (Errea *et al*., 2001). Graft incompatibility symptoms in woody species include bark thickening in the connection region, chlorotic leaves, premature leaf fall, budding delay, vigor differences between the rootstock and scion, excessive stem thickening below, above, or at the point of the graft union. Other symptoms are graft union disruption, reduced vegetative growth, low productivity, and premature plant death (Zarrouk *et al*., 2010; Hartmann *et al*., 2011).

The grafted partners frequently belong to the same species or genus although the use of genetically divergent genotypes is also common. Incompatibility repeatedly occurs in the plum when it is grafted on other Prunus species such as the apricot graft. Different reasons may influence graft success, including the inherent system of cellular incompatibility, the formation of plasmodesmata, vascular tissue connections, and the presence of growth regulators and peroxidases (Usenik *et al*., 2006). Macromolecules (phloem proteins, RNA, and hormones) that are present in the sap phloem might also be important during vascular differentiation in the compatibility process (Pina and Erea, 2005). Different methods for an early detection of graft incompatibility have already been used, including in vitro techniques (Errea *et al*., 2001), isozyme analysis (Fernandez-Garcia *et al*., 2004; Gulen *et al*., 2002), and phenol analysis (Musacchi *et al*., 2000). Such compounds are important to the early growth stages of connections between scion-rootstock combinations since the cell walls of xylem tissues are dynamic structures composed of polysaccharides, phenolic compounds, minerals, and proteins (Herrero *et al*., 2014). Moreover, the presence of phenolic compounds has been identified as an important marker for the evaluation of graft compatibility between scions and rootstocks (Prabpreea *et al*., 2018).

The analysis and recognition of structural phenol diversity are of particular interest because of their physiological roles during the first steps of graft establishment (Usenik *et al*., 2006). The presence of phenols was generally associated with small cells in incompatible combinations, which did not lead to successful unions (Errea *et al*., 2001). Higher concentrations of catechin and epicatechin were found in quince-incompatibility cultivars before the appearance of visible incompatibility symptoms (Musacchi *et al*., 2000). In less compatible apricot combination higher level of flavanols, catechin, and epicatechin, was characteristics (Errea *et al*., 2000).

In several apricot combinations grafted on prunus rootstocks, graft incompatibility resulted in breakdown of the trees at the union years after planting, therefore an early selection process could help in detecting a comparatively compatible combination. Analysis of the phenol content at the graft union can be used as a technique for the estimation of graft incompatibility (Dogra *et al*., 2018).

Several studies have shown that phenolic compounds in incompatible combinations move from vacuole to cytoplasm and cause inhibition of lignification which is required during early stages of establishment of scion–stock connections. The cell wall of xylem vessels are dynamic in nature composed of phenolic compounds (for example, lignins), minerals, polysaccharides and proteins (Liu, 2012; Herrero *et al*., 2014). Plant hormones, especially auxins determine the compatibility of a rootstock-scion combination by interacting with phenolic compounds. Incompatibility has been associated with increased levels of phenolic compounds above the graft union which adversely affect the auxin transport (Errea, 1998). Low auxin concentration in incompatible combinations in turn affect the differentiation of vascular tissues and lignification (Aloni, 2010; Koepke and Dhingra, 2013). All these changes will lead to the formation of weak unions which may cause huge economic losses to the growers. More information about the compounds responsible for inducing graft incompatibility is needed (Gainza *et al*., 2015).

Given the above-mentioned explanations, the current study mainly sought to evaluate the relationship between graft incompatibility and the total phenolic content in some commercial plum cultivars, as well as to determine whether such analysis can be a useful tool for the early detection of graft incompatibility.

MATERIALS AND METHODS

Plant material

This research was conducted at at Golmakan Horticultural Research Station (59° 17' N; 36° 32' E), north east of Iran/Mashhad, with an average altitude of about 1176 m. The mean temperature for growing season was 13.4°C and total seasonal precipitation was 239.7 mm. The nursery soil was sandy loam with low organic matter. Drip irrigation was applied in the nursery. The trees were planted at a spacing of 100 × 10 cm (100.000 trees ha-1) and budded (T-budding technique) 10 cm above the ground level. All rootstocks (apricot and myrobalan) were seedlings, and the samples were taken from 1-year-old plum trees. The content of total phenols above, below, and at the union graft in 10 plum cultivars (i.e., Santarosa, Ghatreh tala, Laroda, Stanley, Dargazi, No. 16, No. 17, Bokhara, Shams, and Simka) grafted on myrobalan and apricot seedling rootstocks was analyzed as well.

Field study

Trees were used one year after grafting for the study. The stem diameters of scions, stocks, and the graft union were measured using a pair of caliper. The units of measurment was to mm.

Phenol extraction

Three trees from each grafting combination were analyzed, and the samples were collected in June. The small sections of the bark above, below, and at the union graft (1 cm above and below the graft union, 1.5 cm in length) were removed with a knife and immediately frozen in liquid nitrogen. Phloem with cambium was used for analysis.

The samples were extracted with a 1.5 ml methanol-acetone-water solution (7:7:1 v/v/v). In a mortar, 50 mg of the plant material was homogenized with a 1.5 ml extraction solution. Next, the samples were centrifuged at 6000Xg for 20 min using a bench centrifuge. Then, the solvents were evaporated in rotary at 40 ^{Ú}C, and the residue was dissolved in 5 ml of deionized water. The extracts were stored at -80 ^{Ú}C until the analysis of the total phenolic content (Mngomba *et al*., 2008). The applied chemical reagents were obtained from Merck Company.

Total phenolic content analysis

The amounts of the total phenol content in pulm cultivar extracts were determined with the Folin-Ciocalteau reagent using the method of Spanos and Wrolastad (1990), as modified by lister and Wilson (2001). To this end, 0.5 ml of Folin-Ciocalteau reagent and 2 ml of Na_2CO_3 (7.55/a, w/v) were added to 100 µl of each sample (three replicates) and then incubated at 45 ^{Ú}C for 15 min. The absorbance of all samples was measured at 620 nm using a SPECTRA max-PLU5384 UV-Vis spectrophotometer. The results were expressed as milligrams of catechol acid equivalent per gram of dry weight (Ganji Moghadam *et al*., 2007).

Statistical analysis

The trial was laid out in a factorial experiment based on completely randomized design with three replications where each replication contained 10 trees. Factors a contains cultivars in 10 levels (Santarosa, Ghatreh tala, Laroda, Stanley, Dargazi, No. 16, No. 17, Bokhara, Shams, and Simka), factor b contains rootstock in 2 levels (myrobalan and apricot seedling) and Factor c contains 3 levels (above, below, and at the union graft). Three replicates of each sample were used for statistical analysis using MSTAT-C, version 1.42. Data were subjected to the analysis of variance, and means were compared by the least significant difference. Differences at $P<0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Significant differences in stem diameters were observed above, below, and at the union graft. The stem diameters below the graft unions were visibly greater than those of above and the union graft on myrobalan rootstock and Santarosa, Dargazi, Bokhara, No. 16, and No.17 cultivars on the apricot rootstock. The unions were thicker than scions and stocks in Ghatreh tala, Shams, Laroda, Stanley, and Simka on the apricot rootstock (Table 1).

In the study of the independent effect of the total phenol content in the union graft, the highest total phenolic content was detected in the below graft union while the lowest content was found in the above graft union. Above and union graft differences were not significant (Figure 1a).

Based on the evaluation of the effect of the union graft in apricot and myrobalan rootstocks, the highest and

Table 1 : Thickness (mm) above, below, and at union graft of different plum cultivars grafted on apricot and myrobalan rootstocks

Graft Combination	Above the Union	At the Union	Below the Union
Apricot rootstock			
Santarosa	6.82*	11.95a	12.37a
Ghatreh tala	8.2c	16.08a	11.57b
Shams	7.24a	9.09a	7.93a
Laroda	5.8b	11.30a	10.95a
Dargazi	7.15c	12.98b	16.18a
Simka	5.16b	11.79a	9.21a
Bokhara	5.9c	10.27b	11.86a
Stanely	6.86a	13.72a	11.11a
No. 16	6.03b	12.13a	14.41a
No. 17	5.03b	11.62ab	18.21a
Myrobalan rootstock			
Santarosa	6.93b	12.71b	20.55a
Ghatreh tala	8.41c	11.57b	16.84a
Shams	6.76b	10.23b	17.74a
Laroda	7.94c	12.16b	16.38a
Dargazi	9.53c	13.49b	18.48a
Simka	8.22c	13.61b	19.02a
Bokhara	7.00c	9.28b	11.31a
Stanely	8.41b	13.67a	16.99a
No. 16	7.18b	10.88b	19.44a
No. 17	6.83b	11.23b	15.11a

Note : *Means with the same letters within a row are not significantly different at P<0.05.

the lowest total phenolic contents were observed in the below graft union on myrobalan and apricot rootstocks, respectively (Figure 1b). The highest total phenolic content was detected in the below graft union of Laroda, Shams, Stanley, Santarosa, and Dargazi cultivars grafted on the myrobalan rootstock whereas the lowest content was found in the below graft union

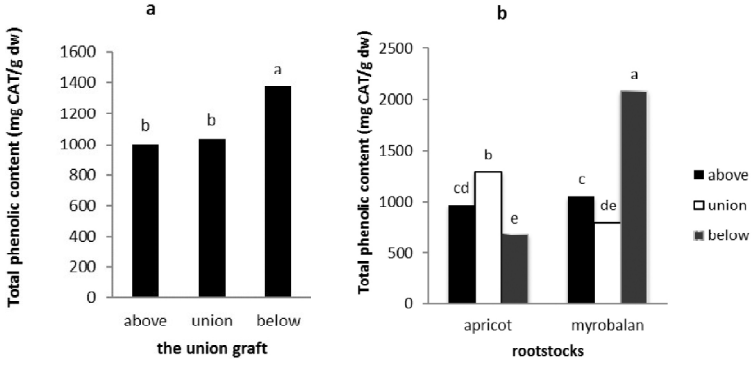

Fig. 1 : Effects of Independent Union Graft (a) and Interaction Rootstocks and Graft Union (b) on the Total Phenolic Content (mg Catechol Acid Equivalent per g of Dry Weight)

of Shams, Laroda, Ghatreh tala, and Santarosa cultivars grafted on the apricot rootstock. The compared differences in the total phenol content in the union and below graft demonstrated the significant accumulation of phenol in the union graft in Santarosa, Ghatreh tala, Laroda, Stanley, Dargazi, Bokhara, Shams, and Simka cultivars grafted on the apricot rootstock while it decreased on myrobalan rootstock (Table 2).

The stem diameters below the graft unions were visibly greater compared to the above and at union graft on myrobalan rootstock and Santarosa, Dargazi, Bokhara, No. 16, and No.17 cultivars on the apricot rootstock. The unions were thicker than the scions and stocks in Ghatreh tala, Shams, Laroda, Stanley, and Simka on the apricot rootstock. The highest total phenolic contents were detected in myrobalan rootstocks while the lowest contents were found in apricot rootstocks. The composition of phenols depends on the genetic constitution of the plant species, and hence, some plants accumulate more than others. These results are in agreement with those of Pina ane Errea (2005) indicating that some apricot cultivars grafted onto a plum rootstock demonstrated only some callus differentiation occurred on cambium and vascular tissues while a large portion of the callus never demonstrated a differentiation. This interrupts vascular connections because of the lack of differentiation that brings discontinuities in the cambium and the formation of a band of

Table 2 : The Amount of the total phenol content (mg gallic acid equivalent per g of dry weight) in above, below, and at the union graft of different plum cultivars grafted on apricot and myrobalan rootstocks

Graft Combination	Above the Union	At the Union	Below the Union
Apricot rootstocks			
Santarosa	1033.79a	1274.88a	441.01b
Ghatreh tala	1051.14b	1905.93a	430.14b
Shams	1010.95a	1424.65a	317.81b
Laroda	810.05a	902.28a	401.82a
Dargazi	1114.15a	1302.28a	747.94a
Simka	1166.21a	1513.24a	839.27a
Bokhara	677.17a	951.59a	555.25b
Stanely	762.56a	1250.23a	698.63a
No. 16	925.15b	1135.16a	1091.33ab
No. 17	807.31b	1347.91a	1204.56a
Myrobalan rootstocks			
Santarosa	611.78b	363.47b	2230.13a
Ghatreh tala	1421.91a	663.93b	1476.86a
Shams	805.47b	1112.32b	2413.69a
Laroda	1378.99b	536.07b	3215.52a
Dargazi	830.14b	449.31c	1221.46a
Simka	1053.88b	763.47b	1789.95a
Bokhara	838.36b	989.04b	1813.69a
Stanely	1120.59b	1114.15b	2291.33a
No. 16	629.23c	1309.59b	1882.19a
No. 17	1828.31a	619.18b	2122.51a

Note : *Means with the same letters within a row are not significantly different at P<0.05.

parenchymatous cells. Based on the findings regarding the evaluation of the independent effect of the total phenol content in the union graft, the highest and lowest total phenolic contents were detected in the below and above graft unions, respectively. The results related to the effect of the union graft on apricot and myrobalan rootstocks, the highest and lowest total phenolic contents belonged to below graft union on myrobalan rootstock and apricot rootstock, respectively. Mngomba *et al*. (2008) reported that the accumulation of phenol deposits at the place of the graft union might inhibit graft compatibility. Usenik *et al*. (2006) also demonstrated that differences in phenol accumulation below and above the graft union might serve as an indicator of incompatibility.

The highest total phenolic content was detected below the graft union of Laroda, Shams, Stanley, Santarosa, and Dargazi cultivars grafted on myrobalan rootstock whereas the lowest content was found in the below graft union of Shams, Laroda, Ghatreh tala, and Santarosa cultivars grafted on apricot rootstock. The comparison of differences in the total phenol content in union and below graft showed the significant accumulation of phenol in the union graft in Santarosa, Ghatreh tala, Laroda, Stanley, Dargazi, Bokhara, Shams, and Simka cultivars grafted on apricot rootstock while it decreased on myrobalan rootstock. In apricot/plum combinations, a high concentration of phenolic compounds was observed in undifferentiated callus at the scion-rootstock interface of plants previously categorized as incompatible (Pina *et al*., 2012), and thus they are involved in the processes of differentiation of vascular tissues (Usenik *et al*., 2006), which is in line with our results. The statistically significant accumulation of phenol in the graft union was ascertained in Santarosa, Ghatreh tala, Laroda, Stanley, Dargazi, Bokhara, Shams, and Simka cultivars grafted on apricot rootstock when compared with the content below the graft union while phenol above the graft union decreased in plum cultivars on myrobalan rootstock. The highest accumulation of phenol in the union graft that can be used as a biochemical marker of graft incompatibility are observed in Ghatreh tala, Shams, and Santarosa on apricot rootstock, which corroborates with the findings of Prabpreea *et al*. (2018), implying that the presence of phenolic compounds has been identified as an important marker for the evaluation of graft incompatibility between scion and rootstocks in the union graft.

CONCLUSION

The early phase of graft incompatibility is complex and needs further evaluation. Phenol analysis is an applicable early sign for the prediction of graft incompatibility, especially when there are new cultivar/ rootstock combinations. The results showed that Ghatreh tala, Shams, and Santarosa on apricot rootstock have the highest graft incompatibility, respectively.

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